Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study

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Summary

Background Sepsis is one of the most common causes of neonatal deaths globally. Most sepsis-related deaths occur in low-income and middle-income countries, where the epidemiology of neonatal sepsis remains poorly understood. Most of these countries lack proper surveillance networks, hampering accurate assessment of the burden of sepsis, implementation of preventive measures, and investment in research. We report results of neonates born in hospital from a multicentre collaboration on neonatal sepsis.

Methods In this cohort study, dedicated research teams prospectively followed up neonates born in one of three tertiary care centres in Delhi, India (Vardhaman Mahavir Medical College, Maulana Azad Medical College, and All India Institute of Medical Sciences [coordinating centre]) and subsequently admitted to the intensive care unit. Neonates were followed up daily until discharge or death. On clinical suspicion, neonates underwent sepsis work-up including blood cultures. The isolated organisms were identified and tested for antimicrobial susceptibility. We defined Gram-negative isolates resistant to any three of five antibiotic classes (extended-spectrum cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and piperacillin-tazobactam) as multidrug resistant.

Findings 13 530 neonates of 88 636 livebirths were enrolled between July 18, 2011, and Feb 28, 2014. The incidence of total sepsis was 14·3% (95% CI 13·8–14·9) and of culture-positive sepsis was 6·2% (5·8–6·6). Nearly two-thirds of total episodes occurred at or before 72 h of life (defined as early onset; 1351 [83%] of 1980). Two-thirds (645 [64%]) of 1005 isolates were Gram-negative including, *Acinetobacter* spp (22%), *Klebsiella* spp (17%), and *Escherichia coli* (14%). The pathogen mix in early-onset sepsis did not differ from that of late-onset sepsis (ie, after 72 h). High rates of multidrug resistance were observed in *Acinetobacter* spp (181/222, 82%), *Klebsiella* spp (91/169, 54%), and *Escherichia coli* (52/137, 38%) isolates. Meticillin resistance prevailed in 61% (85/140) of coagulase-negative staphylococci and 38% (43/114) of *Staphylococcus aureus* isolates. Nearly a quarter of the deaths were attributable to sepsis. The population-attributable risks of mortality were 8·6% in culture-negative sepsis, 15·7% in culture-positive sepsis by multidrug-resistant organisms, and 12·0% in culture-positive sepsis by non-multidrug-resistant organisms.

Interpretation The high incidence of sepsis and alarming degree of antimicrobial resistance among pathogens in neonates born in tertiary hospitals underscore the need to understand the pathogenesis of early-onset sepsis and to devise measures to prevent it in low-income and middle-income countries.

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Introduction

Sepsis is one of the three most common causes of neonatal deaths globally.[1] Most infection-related deaths in the neonatal period occur in low-income and middle-income countries due to poor hygiene and suboptimal practices for infection control. A significant proportion of these deaths are caused by multidrug-resistant pathogens.[2] Despite the massive burden, few high-quality data about neonatal sepsis are available from these countries.[2,3]

The currently available multisite studies on sepsis are from well-established surveillance networks in high-income countries such as the USA,[4] the UK,[5] and Germany.[6] Such infection surveillance networks are a rarity in low-income and middle-income countries;[3] the few available ones have used passive surveillance (eg, the National Neonatal Perinatal Database [NNPD][7] and the Asia-Pacific Neonatal Infections Study [APNIS][8]). Most of the other studies from low-income and middle-income countries are typically from a single site, retrospective, or have relied on routine laboratory reports.[9–12] They often lack rigorous data collection and reporting methods, and run the risk of misclassification and underestimation or overestimation of the incidence of sepsis.[13–15]

The paucity of high-quality data has undermined the recognition of neonatal sepsis as an area of serious concern in public health, the implementation of measures aimed at improvement of health-systems,[3,13,14] and investments in research and innovation in low-income and middle-income countries.[14] In this study, we report data for incidence, profile of organisms, and

Research in context

Evidence before this study

We searched PubMed using the terms "sepsis" and "neonate" (from Jan 1, 2005 to Feb 29, 2016), and limited the search to studies from low-income and middle-income countries. Of the 1460 citations, we included 29 studies reporting data on predominantly (50% or more) neonates born in hospital, and had included at least 20 neonates with sepsis. We excluded studies with a community setting and those reporting data on only single organisms. Most studies were from a single centre (26) and had reported routine microbiology and clinical data (27). Generally, the quality of studies was low. The median number of neonates with sepsis was 84 (IQR 50–143). The incidence of culture-positive sepsis varied from 3·0 per 1000 livebirths to 54·9 per 1000 livebirths (six studies) and case fatality rate varied from 9% to 30% (7 studies). The proportion of early-onset sepsis (onset within 2–4 days after birth; seven studies) ranged from 10·4% to 85·0% of total neonatal sepsis. On enlisting the three most common pathogens in each study, we found *Klebsiella* spp (15 studies), *E coli* (10 studies), and *Staphylococcus aureus* (ten studies) to be the common isolates. *Acinetobacter* spp and group B streptococci were reported as one of three common pathogens in two and five studies, respectively. Pathogen profiles were similar between early-onset and late-onset sepsis (ten studies). The three Gram-negative pathogens—*Klebsiella* spp, *E coli*, and *Acinetobacter* spp—showed a high degree of resistance to commonly used antibiotics such as ampicillin (up to 100%, 100%, and 80%, respectively), cefotaxime (95%, 86%, and 75%), and gentamicin (91%, 79%, and 100%). Carbapenem resistance was reported in *Acinetobacter* spp (0–30%; four studies) and *E coli* (0–15%; four studies); of the five studies reporting data for carbapenem resistance in *Klebsiella* spp, none showed resistance to carbapenems.

Added value of this study

Our study fills a substantial significant gap in the understanding of epidemiology of sepsis in neonates in low-income and middle-income countries. We report a high burden of sepsis among neonates born in tertiary hospitals. Almost two-thirds of sepsis episodes occurred at or before 72 h of life and were caused by pathogens usually associated with nosocomial infections. *Acinetobacter* spp emerged as the most common pathogen. There was a high degree of antimicrobial resistance even to reserve antibiotics such as carbapenems. Approximately half of culture positive neonates died due to sepsis and a quarter of all the deaths were attributable to sepsis.

Implications of all the available evidence

The findings underscore the need to examine the disease biology of early-onset sepsis, including its association with obstetric and neonatal care practices around birth, and to design relevant strategies to prevent infections.

antimicrobial resistance from a prospective cohort study involving three major hospitals in Delhi, India.

Methods

Study design and participants

The Delhi Neonatal Infection Study (DeNIS) collaboration comprises investigators at three tertiary care neonatal units: Vardhaman Mahavir Medical College (VMMC), Maulana Azad Medical College (MAMC), and All India Institute of Medical Sciences (AIIMS; coordinating centre), and one extramural neonatal unit (Chacha Nehru Bal Chikitsalaya) in Delhi. This descriptive cohort study was done among neonates delivered in the tertiary care hospitals (data for Chacha Nehru will be reported separately; appendix p 3) and who required admission to the neonatal intensive care unit (NICU) for any indication during the study period. Neonates requiring rehospitalisation after initial discharge were excluded. The research staff tracked all NICU admissions and enrolled neonates after obtaining informed consent from parents. The research staff prospectively recorded potential maternal risk factors at birth and neonatal factors on a daily basis until discharge or death of the neonates (appendix pp 20–21).

See Online for appendix

The study was approved by the Institutional Ethics Committees of AIIMS, VMMC, and MAMC. Written informed consent was taken from the parents of enrolled neonates.

Procedures

All enrolled neonates were monitored daily for signs and symptoms of sepsis. Sepsis was suspected in the presence of perinatal risk factors or a set of clinical signs as per the Young Infant Study Algorithm.[16] The research nurses obtained blood and, if needed, cerebrospinal fluid samples under strict aseptic conditions and sent them for culture and sepsis screen (appendix p 6) before initiation of any antibiotic therapy. Lumbar puncture was done in all cases of suspected sepsis at one site and only when suspected of meningitis at the other two sites.

The clinical team initiated antibiotics according to the policy of each unit (appendix p 3). The research nurses recorded the age at suspicion of sepsis, investigations done, details of the antibiotics administered, and the clinical course of the baby on a daily basis.

Samples of blood and other body fluids were subcultured after overnight incubation in 5% sheep blood agar (BioMerieux, Marcy l'Etoile, France) and McConkey agar (Oxoid, Hampshire, UK; appendix p 22). Antimicrobial resistance of the isolates was determined as per Clinical and Laboratory Standards Institute guidelines (2011–13).[17–19] Antimicrobial resistance was reported as susceptible, intermediate, resistant, or not tested for each individual antibiotic. Additionally, the Gram-negative pathogens were classified based on their resistance (intermediate or resistant) to various antibiotic

classes: extended-spectrum cephalosporins (any two of ceftazidime, ceftriaxone, or cefotaxime); carbapenems (imipenem or meropenem); aminoglycosides (any one of gentamicin, amikacin, or netilmicin); fluoroquinolones (ciprofloxacin); and piperacillin–tazobactam. In the absence of universally accepted criteria, we defined multidrug resistance as resistance to any three of these five antibiotic classes (adapted from Sievert and colleagues[20]).

Two paediatricians prospectively assigned the diagnosis of sepsis for each suspected episode after reviewing the clinical course, sepsis screen, and culture reports. We used standard definitions of sepsis, adapted from National Healthcare Safety Network (panel, appendix pp 4–5).[21]

Data management and quality assurance

The research staff at each site checked the case record forms daily for accuracy and completion, and the site investigators cross-checked the data weekly. Data were entered in duplicate in an online database developed in Visual Basics as front-end and MS SQL server as back-end with inbuilt range and logical checks.

We put in place a detailed quality assurance system for clinical and laboratory procedures, and data management system (appendix p 7). Detailed standard operating procedures were developed for key processes (abridged versions provided in the appendix pp 20–23). A dry run was carried out for 4 weeks before finalising the case record forms and standard operating procedures. The case record forms were cross-checked by the faculty investigators at the sites as well as at the coordinating centre on a regular basis. Microbiology laboratories used specially procured high-quality culture media and antibiotic disks. Antibiotic disk batches were cross-checked using ATCC strains. As a part of an external quality assurance scheme, the identification and antimicrobial resistance pattern of 10% of isolates were cross-checked at a different participating site.

Statistical analysis

We calculated the incidence of sepsis by dividing the number of neonates with sepsis by the total number of NICU admissions; 95% CIs were calculated using the *cii* command in Stata. The incidence density was calculated as the number of episodes of sepsis per 1000 patient-days or 1000 device-days. Population-attributable risk of mortality for different categories of sepsis was estimated by using the command *csi* in Stata. Statistical analysis was done with Stata 11.2.

Role of the funding source

The scientists of the Indian Council of Medical Research provided valuable inputs in protocol development and data analysis, and reviewed the final manuscript. The corresponding authors have full access to all the data in the study and take final responsibility for the decision to submit for publication.

***Panel:* Definitions used in the study**

Culture-positive sepsis
Isolation of a recognised pathogen from blood, cerebrospinal fluid, or other body fluids in neonates suspected to have sepsis on the basis of clinical features or maternal or perinatal risk factors, along with treatment involving appropriate type and duration of antibiotic therapy. Cases of sepsis with positive culture for coagulase-negative staphylococci were labelled only if the clinical course was suggestive of sepsis and appropriate antibiotic therapy was given.

Culture-negative sepsis
Clinical course suggestive of sepsis or positive sepsis screen, but no pathogen isolated or blood culture not done.

Total sepsis
Number of neonates with culture positive sepsis or culture negative sepsis.

Early-onset sepsis
Occurrence of sepsis at or before 72 h of life.

Late-onset sepsis
Occurrence of sepsis after 72 h of life.

Central-line-associated bloodstream infection
Having a central line (umbilical or non-umbilical) for more than 2 calendar days before the day of onset of blood culture-positive sepsis.

Meningitis
Positive cerebrospinal fluid culture, Gram staining, or neutrophilic leucocytosis, with or without low sugar (less than 50% of plasma glucose level) and high protein content.

Detailed definitions are provided in the appendix.

Results

88 636 livebirths occurred from July 18, 2011, to Feb 28, 2014. 14 779 (16·7%) neonates required NICU admission. After excluding 1249 neonates who were recruited in a concurrent trial, 13 530 (90·0%) were enrolled in the study (9239, 2657, and 1634 at the three sites; figure 1).

The mean birthweight of enrolled neonates was 2211 g (SD 741), and mean gestation was 36·0 weeks (SD 3·4; table 1, appendix p 8). Approximately two-thirds of neonates (8111, 59·9%) were low birthweight, and nearly half (5989, 44·2%) were preterm. A third of enrolled neonates (4708, 34·8%) were born by caesarean section. Although about a quarter (3170, 23·4%) of the mothers received antibiotics within 7 days before delivery, only a small proportion (587, 4·3%) received antibiotics for 48 h or more.

4650 episodes of sepsis were suspected and 7131 cultures were done in 4408 neonates. A final diagnosis of sepsis was assigned in 1980 episodes (1934 neonates). The incidence of total sepsis was 14·3% (95% CI 13·8–14·9) and that of culture-positive sepsis was 6·2% (5·8–6·6; table 2, appendix p 6) of NICU admissions. The incidence density of total and culture-positive sepsis was 24·6 (95% CI 23·6–25·7) and 10·5 (9·8–11·3) per 1000 patient-days, respectively (table 2, appendix p 10). In terms of livebirths, the incidence of total and culture-positive sepsis was 21·8

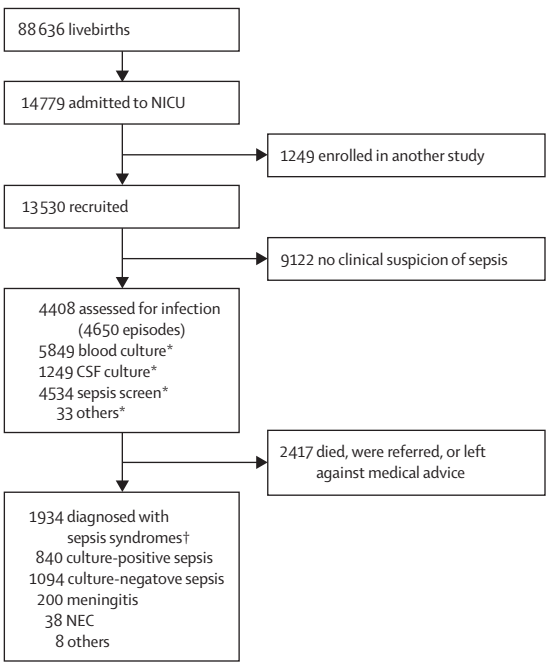

Figure 1: **Study flow**
NICU=neonatal intensive care unit. NEC=necrotising enterocolitis. *Refers to number of cultures done and not the number of neonates. †Not mutually exclusive categories.

	Number of neonates (n=13 530)
Neonatal variables	
Birthweight, g	2211 (741)
Gestation, weeks	36·0 (3·4)
Small for gestation	3641 (26·9%)
Sex	
Boys	7678 (56·7%)
Girls	5852 (43·3%)
Multiple births	1493 (11·0%)
Caesarean delivery	4703 (34·8%)
Major malformations	804 (5·9%)
Positive-pressure ventilation	4187 (30·9%)
Maternal and perinatal variables	
Antenatal corticosteroids	3970/4900 (81·0%)*
Maternal fever within 7 days before delivery	1057 (7·8%)
Maternal antibiotics within 7 days before delivery	3170 (23·4%)
Received for 48 h or more	587 (4·3%)
Per-vaginal examination (≥3)	5209 (38·5%)
Prolonged rupture of membranes (18 h or more)	1958 (14·5%)
Prolonged labour (24 h or more)	173 (1·3%)
Meconium-stained liquor	3073 (22·7%)
Foul-smelling liquor	374 (2·8%)
Neonatal care-related variables	
Intravenous fluids	8928 (66·0%)
Parenteral nutrition	272 (2·0%)
Peripheral arterial line	449 (3·3%)
Any central line	835 (6·2%)
Continuous positive airway pressure	2746 (20·3%)
Mechanical ventilation	1619 (12·0%)
Jaundice requiring phototherapy	3402 (25·1%)
Duration of NICU stay, days	2·7 (1·3–5·5)

Data are mean (SD), median (IQR), or n (%). NICU=neonatal intensive care unit. *Only in neonates below 35 weeks' gestation.

Table 1: **Demographic details of enrolled neonates**

and 9·5 per 1000 livebirths, respectively. Meningitis was detected in 200 (1·5%) neonates, whereas central-line-associated bloodstream infection was diagnosed in 44 episodes of sepsis (9·9 per 1000 central-line days). 1351 (68·2%) of 1980 sepsis episodes—more than two-thirds—were early onset. The incidence of total and culture-positive sepsis varied among the study sites, ranging from 11·9% to 18·9% for total sepsis and 3·6% to 10·5% for culture-positive sepsis (table 2).

Of the total 1005 isolates, about two-thirds were Gram-negative pathogens, the most common being *Acinetobacter* spp, *Klebsiella* spp, *Escherichia coli*, *Pseudomonas* spp, and *Enterobacter* spp (table 3). The predominant Gram-positive pathogens were coagulase-negative staphylococcus, *Staphylococcus aureus*, and *Enterococcus* spp. Group B streptococci were isolated in only a few neonates. The case fatality rate in neonates with sepsis due to Gram-negative pathogens was higher than that of neonates infected with Gram-positive pathogens (59% *vs* 33%). Neonates infected with *Pseudomonas* spp had the highest case fatality rate (table 3).

The pathogen mix in early-onset sepsis did not differ much from that of late-onset sepsis (ie, sepsis after 72 h; appendix p 13). The relative proportion of each pathogen isolated on different days of life was similar (figure 2). The profile of isolates showed substantial variation among the three study sites: the most common isolate was *Acinetobacter* spp (155/576, 27%) in the first site, *Klebsiella* spp (89/359, 25%) in the second site, and coagulase-negative staphylococcus (32/70, 46%) in the third site (appendix p 14).

Most isolated pathogens showed a high degree of antimicrobial resistance, not only to commonly used antibiotics but also to so-called reserve antibiotics such as extended-spectrum cephalosporins and carbapenems (table 4, appendix p 15). A high proportion of *Acinetobacter* spp (181/222, 82%), *Klebsiella* spp (91/169, 54%), and *E coli* (52/139, 38%) was multidrug resistant. Colistin resistance was detected in seven (1%) Gram-negative isolates. Among Gram-positive pathogens, meticillin resistance was detected in 61% (85/140) of coagulase-negative staphylococci and 38% (43/114) of *S aureus*. All the isolates of coagulase-negative staphylococci and *Staphylococcus aureus* were susceptible to vancomycin, but about a quarter of enterococci isolates (13/46, 27%) were resistant. The case fatality rate of sepsis caused by resistant pathogens was only slightly higher than that of sepsis caused by sensitive isolates (table 4).

	Total sepsis	Culture-positive sepsis	Culture-negative sepsis	Meningitis
Incidence*				
Overall (n=13 530)	1934 (14·3%; 13·8–14·9)	840 (6·2%; 5·8–6·6)	1094 (8·1%; 7·6–8·6)	200 (1·5%; 1·3–1·7)
Site 1 (n=9239)	1237 (13·4%; 12·7–14·1)	502 (5·4%; 5·0–5·9)	735 (8·0%; 7·4–8·5)	119 (1·3%; 1·1–1·5)
Site 2 (n=2657)	502 (18·9%; 17·4–20·4)	279 (10·5%; 9·4–11·7)	223 (8·4%; 7·4–9·5)	67 (2·5%; 1·9–3·2)
Site 3 (n=1634)	195 (11·9%; 10·4–13·6)	59 (3·6%; 2·7–4·6)	136 (8·3%; 7·0–9·8)	14 (0·9% 0·5–1·4)
Incidence density†				
Overall (n=80 427)	1980 (24·6; 23·6–25·7)	847 (10·5; 9·8–11·3)	1133 (14·1; 13·3–14·9)	200 (2·5; 2·2–2·8)
Site 1 (n=42 419)	1246 (29·4; 27·8–31·0)	502 (11·8; 10·8–12·9)	744 (17·5; 16·3–18·8)	119 (2·8; 2·3–3·3)
Site 2 (n=21 342)	517 (24·2; 22·2–26·4)	281 (13·2; 11·7–14·8)	236 (11·1; 9·7–12·5)	64 (3·0; 2·3–3·8)
Site 3 (n=16 666)	217 (13·0; 11·3–14·8)	64 (3·8; 2·9–4·9)	153 (9·2; 7·8–10·7)	14 (0·8; 0·4–1·4)
Case fatality rate‡				
Overall	496/1934 (25·6%; 23·7–27·7)	400/840 (47·6%; 44·2–51·0)	96/1094 (8·8%; 7·2–10·6)	102/200 (51·0%; 43·8–58·1)
Site 1	248/1237 (20·0%; 17·8–22·4)	200/502 (39·8%; 35·5–44·3)	48/735 (6·5%; 4·8–8·6)	45/119 (37·8%; 29·1–47·2)
Site 2	226/502 (45·0%; 40·6–49·5)	188/279 (67·4%; 61·5–72·8)	38/223 (17·0%; 12·3–22·6)	56/67 (83·6%; 72·5–91·5)
Site 3	22/195 (11·3%; 7·2–16·6)	12/59 (20·3%; 11·0–32·8)	10/136 (10·4%; 3·6–13·1)	1/14 (7·1 %; 0·2–33·8)

*Among those admitted to neonatal intensive care. Data are number of cases (%; 95% CI). †Data are number of episodes, (number of episodes per 1000 patient-days; 95% CI). ‡Data are number of deaths/number of cases (%; 95% CI).

Table 2: **Incidence and case fatality of neonatal sepsis**

Sepsis was the underlying cause of death in nearly a quarter (505/2106, 24%) of neonates (appendix p 17). The case fatality rate was 26% (496/1934) for all sepsis cases and 48% (400/840) for culture-positive sepsis cases (table 2, appendix p 18). Case fatality rates did not differ between early-onset sepsis (324/1351 [24%] for total sepsis, 258/525 [49%] for culture-positive sepsis) and late-onset sepsis (172/583 [29·5%] and 142/315 [45%], respectively). The attributable risks of mortality were 88·6% in culture-negative sepsis, 97·3% in culture-positive sepsis by multidrug-resistant organisms, and 96·9% in culture-positive sepsis by non-multidrug-resistant organisms; the corresponding population-attributable risks were 8·6%, 15·7%, and 12·0%, respectively (table 5, appendix p 19).

	Number of isolates (n=1005)	Number of deaths (case fatality rate)
Gram negative		
Acinetobacter spp	222 (22%)	130 (59%)
Klebsiella spp	169 (17%)	95 (56%)
Escherichia coli	137 (14%)	83 (61%)
Pseudomonas spp	68 (7%)	53 (78%)
Enterobacter spp	44 (4%)	16 (36%)
Gram positive		
Coagulase-negative staphylococci	150 (15%)	40 (27%)
Staphylococcus aureus	122 (12%)	43 (35%)
Enterococcus spp	56 (6%)	33 (59%)
Group B streptococci	8 (1%)	5 (62%)
Others	29 (3%)	13 (45%)

Data are n (%). See appendix for further details on meningitis and central line associated bloodstream infection.

Table 3: **Profile of bacterial isolates and their case fatality rates**

Discussion

In this large, multisite cohort study, we record a high incidence and case fatality rate of sepsis, accounting for nearly a quarter of deaths in neonates born in tertiary neonatal units in Delhi. Most infections occurred early, and *Acinetobacter* spp emerged as the predominant causative organisms. Most pathogens showed an alarming degree of antimicrobial resistance.

This study deserves attention for three major reasons. First, with more than 80 000 livebirths and 13 500 enrolled neonates, our findings fill a substantial gap in the understanding of the epidemiology of neonatal sepsis in low-income and middle-income countries.[22,23] Second, the data generated are not a byproduct of a passive surveillance as in previous studies (eg, the International Nosocomial Infection Control Consortium [INICC],[24] APNIS,[8] NNPD,[7] and others) but reflect findings of a prospective study with a high degree of methodological rigour. Third, the results would help policy makers to estimate the burden and possible effect of interventions to reduce sepsis-related mortality. The most important findings of our study include early occurrence of sepsis (most episodes occurring at or before 72 h of life), identification of classic late-onset nosocomial pathogens (rather than those typically acquired from the mother) causing early-onset sepsis, *Acinetobacter* spp emerging as the dominant pathogen, and an alarming degree of antimicrobial resistance to even reserve antibiotics.

Most episodes of sepsis occurred at a quite early age, with nearly a quarter of culture-positive sepsis episodes occurring within 24 h of birth and two-thirds within 72 h. This pattern is in sharp contrast with that reported

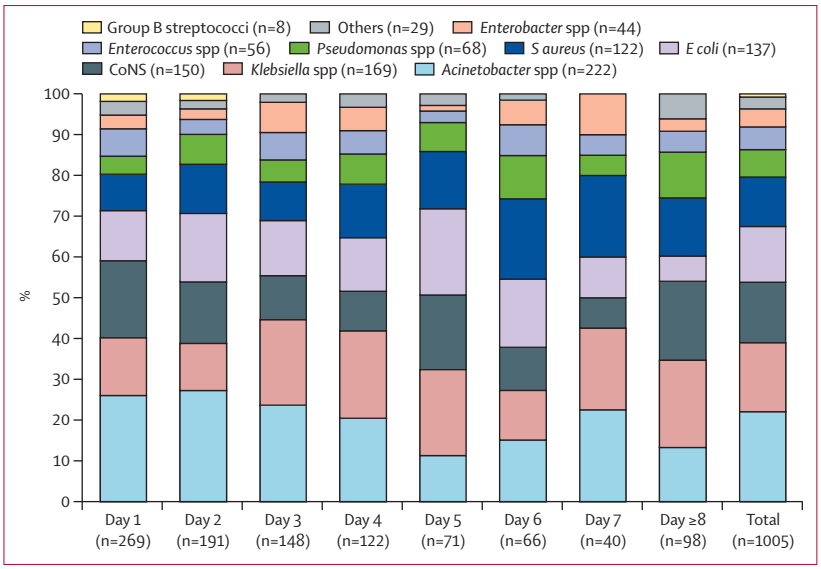

Figure 2: **Profile of pathogens isolated on different days of life**
CoNS=coagulase-negative staphylococci.

from high-income countries, where most infections occur after 72 h of life.[5,25,26] Even studies from other Asian countries such as China[27] and Korea[12] have shown a predominance of late-onset sepsis. Nearly half of neonates with culture-positive sepsis in our study died despite early detection and appropriate supportive care. This finding probably does not result from high antimicrobial resistance, in view of the almost identical case fatality rates between multidrug-resistant and non-multidrug-resistant pathogens (table 4). Unusually high virulence of pathogens could be one possible reason. Traditionally, the common pathogens implicated in early-onset sepsis include *E coli*, group B streptococci, *Listeria monocytogenes*, and *Enterococcus* spp. The findings of our study, however, contrast with this notion as well as reports from high-income countries.[5–7,14] *Acinetobacter* spp, coagulase-negative staphylococci, and *Klebsiella* spp, usually recognised as nosocomial pathogens, were the dominant pathogens even in neonates with early-onset sepsis. Group B streptococci were isolated rarely. Findings from a few other studies with smaller sample size from India have also shown similar results.[9,28] The high rate of early-onset sepsis and the apparent dominance of so-called nosocomial-type pathogens in early-onset sepsis could possibly be due to ultra-early horizontal transmission from delivery rooms and NICUs,[13,14,29] or vertical transmission from the maternal genital tract colonised with these pathogens after unhygienic personal and obstetric practices.[30,31]

Acinetobacter spp (22%) emerged as the most common isolates in our cohort. Although a few recent studies from India[9,10,30,31] and other low-income and middle-income countries did report it as one of the isolates,[11,27,32] none has so far reported such a degree of dominance. Of the 1248 isolates reported by the NNPD Network, *Klebsiella* spp (32%) were the leading pathogens, with the proportion of *Acinetobacter* spp being a low 3%.[7] Are we witnessing a growing scourge of *Acinetobacter* spp infections in India's tertiary neonatal units?[31] In view of the high degree of antimicrobial resistance—including resistance to carbapenems (78·3%)—and the high case fatality rate (59%) in our cohort, the increasing burden of *Acinetobacter* sepsis might pose a formidable threat in coming years. *Acinetobacter baumanii*, the usual dominant species, is capable of rapid clonal expansion in face of selection pressure by broad-spectrum anti-

	Number of resistant isolates	CFR in culture-positive sepsis due to resistant pathogens	CFR in culture-positive sepsis due to sensitive pathogens
Gram negative			
Acinetobacter spp (n=222)			
ES cephalosporins	85/222 (38%)	59/85 (69%)	71/137 (52%)
Carbapenems	174/222 (78%)	106/174 (61%)	24/48 (50%)
MDR	181/222 (82%)	112/181 (62%)	18/41 (44%)
Klebsiella spp (n=169)			
ES cephalosporins	105/169 (62%)	57/104 (55%)	38/65 (58%)
Carbapenems	59/169 (35%)	36/59 (61%)	59/110 (54%)
MDR	91/169 (54%)	52/91 (57%)	43/78 (55%)
Escherichia coli (n=137)			
ES cephalosporins	65/137 (47%)	40/64 (63%)	43/73 (59%)
Carbapenems	21/137 (15%)	12/21 (57%)	71/116 (61%)
MDR	52/137 (38%)	30/52 (58%)	53/85 (62%)
Pseudomonas spp (n=68)			
ES cephalosporins	32/68 (47%)	29/32 (91%)	24/36 (67%)
Carbapenems	21/68 (31%)	19/21 (90%)	34/47 (72%)
MDR	13/68 (19%)	11/13 (85%)	42/55 (76%)
Enterobacter spp (n=44)			
ES cephalosporins	20/44 (45%)	6/20 (30%)	10/24 (42%)
Carbapenems	9/ 44 (20%)	4/9 (44%)	12/35 (34%)
MDR	22/44 (50%)	8/22 (36%)	8/22 (36%)
Gram positive			
Coagulase-negative staphylococci (n=150)			
Meticillin	85/140 (61%)	23/85 (27%)	14/55 (25%)
Vancomycin	0/138	..	36/138 (26%)
Staphylococcus aureus (n=122)			
Meticillin	43/114 (38%)	16/43(37%)	22/71 (31%)
Vancomycin	0/114	..	38/114 (33%)
Enterococcus spp (n=56)			
Meticillin	11/14 (79%)	10/11 (91%)	2/3 (67%)
Vancomycin	13/48 (27%)	9/13 (69%)	20/35 (57%)

Data are n/N (%); there are variations in denominators in few cells as antibiotic sensitivity testing for all drugs was not done. CFR=case fatality rate. ES=extended-spectrum. MDR=multidrug resistance (ie, I [intermediate] or R [resistant] to on drug in three of the following classes: ES cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, and piperacillin-tazobactam).

Table 4: **Case fatality rates among common pathogens by their antimicrobial resistance pattern**

	Died (n=2103)	Survived (n=85 033)	Relative risk (95% CI)	Attributable risk percentage (95% CI)	Population-attributable risk
No sepsis	1457 (69·3%)	83 817 (98·6%)	1·0	..	..
Culture-negative sepsis	158 (7·5%)	894 (1·0%)	8·79 (7·55–10·2)	88·6% (86·7–90·2)	8·6%
Culture-positive sepsis by sensitive organisms (MDR negative or cefoxitin sensitive)	207 (9·8%)	165 (0·2%)	32·6 (29·34–36·13)	96·9% (96·6–97·2)	12·0%
Culture-positive sepsis by resistant organisms (MDR positive or cefoxitin resistant)	281 (13·4%)	157 (0·2%)	37·5 (34·43–40·94)	97·3% (97·1–97·5)	15·7%

Resistance defined as multidrug resistant (for Gram-negative organisms) or cefoxitin resistant (for Gram-positive organisms). For the purpose of calculating population-attributable risk, we used all livebirths as the denominator and assumed that the remaining neonates who did not get admitted to neonatal intensive care (and therefore not enrolled in the study) did not develop sepsis.

Table 5: **Sepsis categories and their attributable and population-attributable risk of mortality**

biotics.[33–35] This property might have led to its proliferation and subsequent dominance in our cohort after sporadic entry in the NICUs. Its endemicity is further facilitated by suboptimal infection control practices including inadequate hand hygiene compliance in low-income and middle-income settings.[14,34,36]

Our results are consistent with a high degree of antimicrobial resistance documented in other reports from India.[9,30,37] The high prevalence of resistance to extended-spectrum cephalosporins, carbapenems, and the emerging resistance to colistin in the three most common Gram-negative isolates (*Acinetobacter* spp, *Klebsiella* spp, and *E coli*) make the choice of antibiotics extremely difficult. We used classes of higher-spectrum antibiotics (carbapenems, piperacillin–tazobactam) to define multidrug resistance rather than the WHO-recommended first-line options such as ampicillin, gentamicin, and cefotaxime.[8,29] Our definition, therefore, includes the most severe form of multidrug resistance. The high rates of multidrug resistance (40–81%)—despite such a rigorous definition—in the three common Gram-negative bacteria threaten the return of preantibiotic era in Indian NICUs.[5,37,38] This frequent multidrug resistance in common pathogens raises the possibility of cross-transmission of mobile genetic elements that are able to jump across genera, including commensals.[39] In preliminary results from our network, the presence of NDM-1 has been documented in nearly a quarter of *Acinetobacter* spp and three-quarters of *Klebsiella* spp among the pool of carbapenem-resistant strains (data not shown).

Although our antimicrobial resistance results are alarming, the more ominous finding that has implications for policy makers is the risk of mortality attributable to culture-positive sepsis in general (table 5). The population-attributable risk in culture-positive sepsis by multidrug-resistant organisms was only slightly higher than that of sepsis by non-multidrug resistant organisms (15·7% *vs* 12·0%). If resistance to only aminoglycosides and cephalosporins (for Gram negative) or cefoxitin (for Gram-positive organisms) is considered, the corresponding population-attributable risk would be 11·8% for sepsis caused by resistant isolates and 15·8% for sepsis caused by sensitive isolates (appendix p 19). The high risk of mortality for sensitive isolates is puzzling, given that inappropriate choice of empirical antibiotics in the first 48–72 h after suspicion of sepsis (ie, until culture reports are available) is unlikely to affect mortality. The results call for a rethink among different stakeholders[3,40] to focus equally, if not more, on strategies to prevent sepsis by having a better understanding of the pathogenesis, along with addressing antimicrobial resistance in intensive care units from low-income and middle-income countries.

Our study has several limitations. First, there was considerable heterogeneity in infrastructure, manpower, practices, and patient profile, which might have masked interinstitutional differences in epidemiology of sepsis. Second, we did not separately record pneumonia, including ventilator-associated pneumonia, because of practical difficulties. Third, we may have overestimated the incidence of coagulase-negative staphylococci, because its diagnosis was not based on two simultaneous blood cultures. However, we followed stringent, protocol-driven practices for skin preparation before obtaining blood culture specimens, and the diagnosis of sepsis caused by coagulase-negative staphylococci was made only after detailed review of clinical course. Fourth, the incidence of sepsis (particularly late-onset sepsis) might have been underestimated because the neonates were not followed up after discharge from the hospital.

Our study highlights the need to undertake research to understand the pathogenesis of early-onset sepsis and to devise measures to prevent related morbidity and mortality. The findings also serve as a yet another wake-up call for global action to curb the escalating menace of antimicrobial resistance.

Contributors

All members of the writing committee contributed to the design and implementation of the study, analysis and interpretation of the data, and drafting of the report. The investigators had an opportunity to critically review results and contribute to the process of finalisation of the report. The writing committee vouches for accuracy and integrity of the work, and accepts full responsibility for the content of the paper.

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Declaration of interests

Members of the writing committee declare no competing interests.

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